INTRODUCTION In rheumatoid arthritis, the role of autoimmune mechanisms is well documented. However, clinical and experimental evidence also strongly supports an involvement of the nervous system. The patients are often affected in a symmetric, neurotome-like fashion. Hemiplegic patients who develop rheumatoid arthritis show little or no signs of inflammation on the paralysed side. Animal studies suggest that the degree of joint inflammation is related to the density of nerve fibres in the joint; sensory and/or autonomic denervation attenuates the severity of arthritis. These observations indicate that the peripheral nervous system regulates the degree of systemic arthritis by release of neuronal mediators with local effects.

Among the neuronal mediators with known effects on inflammation, the sensory neuropeptides substance-P (SP) and calcitonin gene-related peptide (CGRP) are best documented. In the search for other neuronal messengers which may be involved in inflammation, considerable interest has recently focused on opioid peptides i.e. enkephalins, endorphins and dynorphins. Endogenous opioid peptides and exogenous opiate alkaloids have been found to affect the function of granulocytes, lymphocytes, monocytes and macrophages. These effects include modulation of chemotaxis, phagocytosis and antibody production. A few animal trials with opioid analogues have shown significant anti-inflammatory effects.

This study was undertaken to investigate the occurrence and co-expression of sensory neuropeptides and opioids in ankle joints in adjuvant arthritis by means of immunohistochemistry.

METHODS Ten female Lewis rats weighing 230-250g. were inoculated with heat killed Mycobacterium butyricum (50 µl, 10 mg/ml) under pentobarbitone anesthesia. Ten control animals received similar injections with 50 µl vehicle (paraffin oil). After three weeks of onset of arthritis, in vivo perfusion-fixation with Zamboni’s buffered paraformaldehyde was performed under anesthesia and the ankle joints were removed. The joint specimens were demineralized in 4% EDTA in cacodylate buffer. Tissues were embedded in buffered 20% sucrose and sectioned (15 µm) on a Leitz cryostat. The indirect immunofluorescence method was used for immunohistochemistry. Briefly, sections were incubated with primary antisera (Peninsula laboratories, St. Helens, UK) for SP, CGRP, methionine-enkephalin (ME) or leucine-enkephalin (LE). Fluorochrome staining was done with Cy2-conjugated avidin for visualization of the immunoreaction. Double staining was performed by first staining with SP or CGRP using Cy2-conjugated avidin, incubating with avidin and biotin blocking solutions, then staining with ME or LE using another fluorochrome, Cy3-conjugated avidin. Slides were viewed under an epifluorescence microscope (Eclipse E800, Nikon, Tokyo, Japan).

RESULTS Nerve fibres immunoreactive to ME and LE were identified in the synovium, periosteum, bone marrow and cortical bone of both controls and arthritic rats. However, the number of positively-stained nerve fibres was much greater in the arthritic rats compared to controls. In the hypertrophic synovium and periosteum of arthritic rats, a large number of thin varicosc nerve fibres containing ME and LE were identified in the walls of blood vessels, but also non-vascular nerve fibres were present. An abundance of nerve fibres immunoreactive to ME and LE was found in the erosive bony lesions of distal tibia and talus of arthritic rats. Quite a number of nerve fibres showed co-expression of opioids and sensory neuropeptides. Although the majority of these nerve fibres were perivascular, many non-vascular nerve fibres were also seen, mostly in the hypertrophic synovium and periosteum. It is probable that these nerve fibres mediate modulatory effects on inflammatory cells, through release of opioids and sensory neuropeptides.

Peptides from the opioid and sensory neuropeptide families are present in peripheral nerves, often in co-existence as was observed in our study. Since opioids and sensory neuropeptides have different modes of action, it is likely that they modulate the inflammation through different mechanisms, albeit expressed together.

Although the existing literature supports the notion of a physiological role of endogenous opioids in inflammation, evidence of cross-talk between opioids and sensory neuropeptides is still sparse. Notably, opioid receptors have been found on sensory nerve terminals of joints. The anti-inflammatory effects of opioids is partly due to enhanced expression of opioid peptides and opioid receptors on sensory nerves. Activation of this opioid system probably enhances the release of pro-inflammatory sensory neuropeptides.

The observed neuronal up-regulation and co-expression of opioids and sensory neuropeptides in inflamed ankle joints opens the possibility of designing new drugs for neuronal therapy in inflammatory joint disease.

DISCUSSION This study shows an increased expression of neuronal opioids in arthritic joints of rats. Quite a number of nerve fibres showed co-expression of opioids and sensory neuropeptides. Although the majority of these nerve fibres were perivascular, many non-vascular nerve fibres were also seen, mostly in the hypertrophic synovium and periosteum. It is probable that these nerve fibres mediate modulatory effects on inflammatory cells, through release of opioids and sensory neuropeptides.

Conclusions Opioid receptors and sensory neuropeptides are present in the joint. The co-localization of opioids and sensory neuropeptides suggest that the peripheral nervous system regulates the degree of joint inflammation by releasing neuronal mediators with local effects.

CO-EXPRESSION OF OPIOID AND SENSORY NEUROPEPTIDES IN ADJUVANT ARTHRITIS

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